migrating a four ways Holliday junction. The initiation of the repair process requires partial replisome disassembly via the departure of the replicative helicase.

1883-Plat
Dynamics of DNA Mismatch Repair Initiation Complex Revealed by Single Molecule Fluorescence
Ruoyi Qiu, Keith Weninger.
North Carolina State University, Raleigh, NC, USA.
The integrity of the genetic information is dependent on the fidelity of DNA replication and several DNA repair processes. Among these repair systems, DNA mismatch repair (MMR) is responsible for correcting base-base mismatches and small nucleotide insertion/deletion (IDL) mispairs that arise from polymerase misincorporation, elevating fidelity of replication 50-1000 fold. MMR is initiated when MutS binds to mismatched bases on dsDNA. The communication between the mismatch site and a distal strand discrimination signal is required for removing the mismatch from the newly synthesized strand. MutS-MutL-heteroduplex ternary complex is thought to play an key role in the coupling of these two sites on DNA. We used single molecule fluorescence resonance energy transfer (smFRET) to characterize conformational changes in this ternary complex through the process of mismatch recognition, MutS-MutL interaction and large MutS-MutL assembly formation on DNA. We found that the sliding clamp formation of MutS is inhibited by MutL interaction. The initial MutS-MutL complex stays at the mismatch site and recruits more MutS and MutL to form a large protein assembly. The structural information revealed by our single molecule measurements provides constraints for modeling the mechanism of MMR in the initiation stage.

1884-Plat
Dynamics of Site Switching in DNA Polymerase
Rajan Lamichhane, Svitlana Y. Berezhna, Edwin Van, der Schans, David P. Millar.
The Scripps Research Institute, La Jolla, CA, USA.
DNA polymerases replicate DNA by catalyzing the template-directed polymerization of deoxynucleoside triphosphate (dNTP) substrates onto the 3′ end of a growing DNA primer strand. Many DNA polymerases also possess a separate 3′-5′ exonuclease activity that is used to remove misincorporated nucleotides from the nascent DNA (proofreading). The polymerase (pol) and exonuclease (exo) activities are spatially separated in different enzyme domains, indicating that a mechanism must exist to transfer the growing primer terminus from one site to the other. Here we report a single-molecule Förster resonance energy transfer (smFRET) system that directly monitors the movement of a DNA substrate between the pol and exo sites of DNA polymerase I Klenow fragment (Pol I KF). FRET trajectories recorded during the encounter between single polymerase and DNA molecules reveal that DNA can channel between the pol and exo sites in both directions while remaining bound to the enzyme (intramolecular transfer). In addition, it is evident that DNA can also dissociate from one site and rebind at the other (intermolecular transfer). Rate constants for each pathway have been determined by dwell-time analysis for a model primer/template containing a terminal G•G mispair, revealing that intramolecular transfer is the faster of the two pathways. Surprisingly, the mispaired primer terminus accesses the exo site more frequently when dNTP substrates are also present in solution, which is expected to enhance proofreading. These results explain how the separate pol and exo activities of Pol I KF are physically coordinated to achieve efficient proofreading. The results also suggest an expanded role for nucleotides during polymerase function.

1885-Plat
Coupling of Hexameric Helicase and DNA Polymerase in T7 Replisome during DNA Replication
Manjula Pandey, Smita S. Patel.
Robert Wood Johnson Medical School-UMDNJ, Piscataway, NJ, USA.
Bacteriophage T7 replisome is one of the simplest replication machinery and we study the complexities of replication mechanism exploring this system. T7 gene 4 protein (gp4) assembles as a hexameric ring-shaped helicase with translocation polarity of 5’ to 3’ on ssDNA and duplex DNA unwinding during DNA replication. This protein also has a primase domain that generates short RNA primers for priming lagging strand DNA synthesis. It is known that T7 helicase coordinates with T7 DNA polymerase (T7gp5 -Thioredoxin of E. coli) in replisome to catalyze both leading and lagging strand DNA synthesis. However, functional and the physical extent of this relationship is not understood well. We have analyzed chemical coupling and physical proximity of these two motors during DNA replication in this study. Long standing question in the field has been that how does helicase couple the chemical energy of nucleotide hydrolysis for its translocation during DNA replication? This question could not be answered reliably so far due to the complexity and incompatibility of the NTP hydrolysis and helicase translocation activities. using pre-steady state kinetics approach combined with a novel coupling assay we have tackled this question. This is first ever report for the chemical step size determination for the helicase in replisome. Fine details of the energy coupling mechanism of the helicase in replisome are also revealed in these results. Second part of the study demonstrated the relative position of helicase and DNA polymerase in the replisome at the fork during DNA replication.

1886-Plat
Solution Scattering Studies of Large Serine Recombinase-DNA Complexes
Karen Rutherford, Kushol Gupta, Gregory Van Duyne.
University of Pennsylvania, Philadelphia, PA, USA.
Large serine recombinase (LSR) proteins are emerging as desirable tools in targeted gene therapy due to their ability to catalyze the integration of any target DNA into a host genome in a highly specific and unidirectional manner. They have long been shown to mediate the integration and long-term expression of target genes in human stem cell and lung and liver cancer cell lines. LSRs consist of a catalytic domain, containing the active site serine, and a large C-terminal domain responsible for DNA binding. The LSR protein recognizes unique attachment sites in the target and host DNA which confer the high specificity of LSR mediated recombination. Based on current models, recombination is completed when two subunits of a tetrameric recombinase complex undergo a 180° rotation, generating new attachment sites that discourage excision of the target DNA. However, there are currently no published structures of a LSR protein alone or bound to DNA to understand the mechanism of this exquisite directionality. We are close to completing the first crystal structure of a LSR bound to DNA which will provide insight into the high specificity of the LSR-DNA interaction. In addition, we have employed small-angle x-ray scattering (SAXS) and small-angle neutron scattering (SANS) techniques to determine low resolution structures of the LSR-DNA complex intermediates from the integration pathway. Detailed structural models of these molecular intermediates are essential in determining how this recombination reaction occurs.

1887-Plat
Observation of Synapse Rotation of Engineered Recombinase HIN-H107Y
Botao Xiao[1], Reid C. Johnson[2], John F. Marko[3].
[1]Harvard University, Boston, MA, USA, [2]University of California, Los Angeles, Los Angeles, CA, USA, [3]Northwestern University, Evanston, IL, USA.
Site-specific recombination promotes integration, excision or inversion of DNA strands during gene transcription and chromosome replication. The recombination process generally involves the formation of synaptic complexes, DNA cleavage and rejoining, though the specific mechanism of each enzyme varies. The Hin recombinase is a member of the serine recombinase family which has an active serine residue that can catalyze the DNA cleavage and re-ligating. Hin functions to invert a 900 bp DNA segment between two hin-binding (hix) sites within the Salmonella chromosome that contains a promoter for downstream flagellar genes. The inversion requires the auxiliary protein Fis and two Fis-binding sites on the targeted DNA segment, which assemble together with hix sites and Hin to form an invertasome structure. The assembly is stimulated by the protein HU. A class of engineered Hin mutants Hin-H107Y (H107Y) is able to assemble oligonucleotide substrates containing hix sites into stable synaptic complexes that catalyze recombination without Fis and HU. Structural data suggest that Hin share with gamma-delta resolvase a similar helix-turn-helix domain at the 40-50 amino acid residues. We hypothesized that H107Y could act like resolvase to form four subunits synapse, cleave both strands of DNA within the center of hix site, rotate and rejoin. We therefore used single DNA assays to analyze the dynamics associated with H107Y catalyzed strand exchange.
Enzyme-DNA binding and synapse formation was observed with a single DNA containing two hix sites. We further obtained data for subunits rotation and re-ligation from a braiding assay which consisted of double DNAs each having a single hix site, indicating that the rotation strand exchange mechanism observed recently for Bxb1 Int is also possessed by Hin.

1888-Plat
Investigating DNA Replication in Escherichia Coli on the Single Cell Level Utilizing Microfluidics and Single-Molecule Fluorescence Microscopy
Charl Moolman[1], Sriram T. Krishnan[1], Jacob W.K. Kerssemakers[1], Susanne Hage[1], Rodrigo Reyes-Lamothe[2], David Sherratt[3], Nynke H. Dekker[1].
[1]Delft University of Technology, Delft, Netherlands, [2]McGill University, Montreal, QC, Canada, [3]University of Oxford, Oxford, United Kingdom.